Platelet-Derived Growth Factor Stimulates Glucose Transport in Skeletal Muscles of Transgenic Mice Specifically Expressing Platelet-Derived Growth Factor Receptor in the Muscle, but It Does Not Affect Blood Glucose Levels

Tomoyuki Yuasa,1 Rei Kakuha,1,2,3 Kazuhiro Kishi,1 Toshiyuki Obata,1 Yasuo Shinohara,2,3 Yoshimi Bando,4 Keisuke Izumi,4 Fumiko Kajiura,5 Mitsuru Matsumoto,5 and Yousuke Ebina1

Insulin stimulates the disposal of blood glucose into skeletal muscle and adipose tissues by the translocation of GLUT4 from intracellular pools to the plasma membrane, and consequently the concentration of blood glucose levels decreases rapidly in vivo. Phosphatidylinositol (PI) 3-kinase and Akt play a pivotal role in the stimulation of glucose transport by insulin, but detailed mechanisms are unknown. We and others reported that not only insulin but also platelet-derived growth factor (PDGF) and epidermal growth factor facilitate glucose uptake through GLUT4 translocation by activation of PI 3-kinase and Akt in cultured cells. However, opposite results were also reported. We generated transgenic mice that specifically express the PDGF receptor in skeletal muscle. In these mice, PDGF stimulated glucose transport into skeletal muscle in vitro and in vivo. Thus, PDGF apparently shares with insulin some of the signaling molecules needed for the stimulation of glucose transport. The degree of glucose uptake in vivo reached ~60% of that by insulin injection in skeletal muscle, but blood glucose levels were not decreased by PDGF in these mice. Therefore, PDGF-induced disposal of blood glucose into skeletal muscle is insufficient for rapid decrease of blood glucose levels. Diabetes 53: 2776–2786, 2004

Insulin is a unique hormone that decreases the concentration of blood glucose rapidly in vivo. The insulin-stimulated disposal of blood glucose into skeletal muscle and adipose tissue occurs by the translocation of GLUT4 (1–4). The major causes of type 2 diabetes seem to be failures of insulin action and/or insulin secretion (5). Despite extensive related studies, the signaling pathway concerning insulin-stimulated glucose transport is not fully understood. Insulin receptor substrates (IRSs) are phosphorylated by insulin receptor kinase, and consequently phosphatidylinositol (PI) 3-kinase is activated by binding with the phosphorylated IRSs. The activation of PI 3-kinase is essential for insulin-stimulated GLUT4 translocation and glucose uptake (6–8). The serine/threonine protein kinase Akt binds to lipid products of PI 3-kinase and is thought to play a significant role in this pathway (9–14), but the results are controversial (15). The participation of atypical protein kinase C (PKC; i.e., PKCa and -c) in glucose uptake, which is also activated by polyphosphoinositides and 3-phosphoinodide–dependent protein kinase 1, has been proposed, although the role of atypical PKC also seems to be controversial (16–18). An alternative parallel pathway, which involves the tyrosine phosphorylation of c-Casitas B-lineage lymphoma (c-Cbl) and the translocation of the c-Cbl/c-Cbl–associated protein complex to lipid raft subdomains of the plasma membrane, has been noted in glucose transport by insulin (19).

On the other hand, we and others reported that platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) as well as insulin facilitate GLUT4 translocation and consequently glucose uptake through the activation of PI 3-kinase and Akt in cultured cells (20–26). However, several investigators have reported opposite results (27–32). These findings raise questions as to whether PDGF stimulates glucose uptake in skeletal muscle, which is a major target of insulin, in vivo and also as to whether PDGF decreases blood glucose levels. To elucidate the molecular mechanisms involved in the lowering effects on blood glucose levels by insulin, it is useful to compare the signaling mechanisms concerning the glucose uptake stimulated by insulin with those stimulated

From the 1Division of Molecular Genetics, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan; the 2Faculty of Pharmaceutical Sciences, University of Tokushima, Tokushima, Japan; the 3Institute for Genome Research, University of Tokushima, Tokushima, Japan; the 4Molecular and Environmental Pathology, School of Medicine, University of Tokushima, Tokushima, Japan; and the 5Division of Molecular Immunology, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan.

Address correspondence and reprint requests to Yousuke Ebina, Division of Molecular Genetics, Institute for Enzyme Research, the University of Tokushima, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan. E-mail: ebina@ier.tokushima-u.ac.jp.

Received for publication 30 April 2004 and accepted in revised form 3 August 2004.

© 2004 by the American Diabetes Association.
by PDGF. We generated transgenic mice that specifically express the PDGF receptor in skeletal muscle and examined both the signaling mechanisms of glucose transport in skeletal muscle in vitro and in vivo and the effects on blood glucose levels.

**RESEARCH DESIGN AND METHODS**

Recombinant human PDGF-BB (PDGF-B chain homodimer) and human regular insulin were purchased from Austral Biologicals (San Ramon, CA) and Novo Nordisk ( Bagsværd, Denmark), respectively. Polyclonal antibody against PDGF-β receptor (P-20: sc-330), polyclonal antibody against insulin receptor-β (C-19: sc-711), polyclonal antibody against the p110α subunit of PI 3-kinase (S-19: sc-692), and monoclonal antibody against phosphotyrosine (PY99: sc-7020) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antibody against phospho-specific Akt (Ser473) was purchased from Cell Signaling (Beverly, MA). Monoclonal antibody against β-tubulin was purchased from Seikagaku (Tokyo). Secondary horseradish peroxidase–conjugated goat anti-mouse IgG and anti-rabbit IgG were purchased from Amersham Life Science (Boston, MA). Dye-labeled secondary antibodies were purchased from Jackson Immunoresearch Laboratory (West Grove, PA). Primary antibodies were purified from rabbit and goat tissues preserved in liquid nitrogen and then homogenized in ice-cold homogenization buffer (25 mmol/l Tris-HCl, pH 7.4, 1% Nonidet P-40, 1 mmol/l sodium orthovanadate, 10 mmol/l EGTA, and 1 mmol/l phenylmethylsulfonyl fluoride) with a Polytron (Hanna Instruments, Waltham, MA). Dye-labeled secondary antibodies were purchased from Molecular Probes, Invitrogen (Carlsbad, CA), and poly(A) tailing of cDNA (kindly provided by J. Chamberlain, University of Washington) (35), human PDGF-β receptor cDNA (kindly provided by X. Williams, University of California) (36), and rabbit β-globin poly(A)+ signal from plasmid pUC19 vector (37) were subcloned into the plasmid pUC19 vector. The expression unit, which was excised from the resulting pUC19–muscle creatine kinase–human PDGF-β receptor plasmid by digestion with KpnI, was microinjected into fertilized eggs of C57BL/6J mice (Biotechnology Group, Japan SLC, Shizuoka, Japan). The presence of the transgene in founder mice was assessed by Southern blot analysis.

**Animals and genotyping.** All animals were housed in specific pathogen-free facilities on a 12-h light/dark cycle and were fed standard rodent diet ( Oriental Yeast, Tokyo, Japan). All genotyping was performed using Southern blot analysis and genomic DNA isolated from the tail tip of 4-week-old mice. All experiments in this study conformed to the guidelines for the care and use of laboratory animals of the School of Medicine, University of Tokushima.

**Southern and Northern blot analysis.** Mouse genomic DNA (1 μg) purified from mouse tissues stored in liquid nitrogen was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), and poly(A)+ RNAs were prepared from total RNA by oligotex (Takara Shuzo, Kyoto, Japan) according to the manufacturer’s instructions. Poly(A)+ RNAs (3 μg) were fractionated on 1% denaturing agarose gels and then transferred to nitrocellulose membrane. Both membranes were hybridized to random-primer-32P-labeled full-length, human PDGF-β receptor cDNA probes (38). After washing with wash buffer, the membrane was subjected to autoradiography.

**Immunoprecipitations and Western blot analysis.** Mice were fasted for 16 h and then anesthetized by giving an intraperitoneal administration of sodium pentobarbital (65 mg/kg body wt, nembutal injection; Dainippon Pharmaceutical, Osaka, Japan). Striated muscles were removed, preserved in liquid nitrogen, and then homogenized in ice-cold homogenization buffer (25 mmol/l Tris-HCl, pH 7.4, 1% Nonidet P-40, 10 mmol/l sodium orthovanadate, 10 mmol/l sodium pyrophosphate, 100 mmol/l sodium fluoride, 10 mmol/l EDTA, 10 mmol/l EGTA, and 1 mmol/l phenylmethylsulfonyl fluoride) with a Polytron PT10/35 homogenizer (Kinematica, Lucerne, Switzerland). Samples were allowed to solubilize on the rotator for 45 min at 4°C, and unwanted matter was removed by centrifugation at 16,100g for 20 min at 4°C. Supernatant fluid was collected and its protein concentration determined using the Bradford method (protein assay; Bio-Rad Laboratories, Richmond, CA), with BSA as a standard. Equal amounts of protein were precipitated with a specific antibody and protein A-Sepharose CL-4B (Amersham Biosciences, Uppsala, Sweden). The immunoprecipitates were washed four times with wash buffer (0.9% PBS, pH7,4, 0.9% Nonidet P-40, 0.09 mol/l sodium orthovanadate, 0.5 mol/l NaCl).
Extracts from the immunoprecipitates or equal amounts of protein were resolved by SDS-PAGE and transferred by electroblotting onto nitrocellulose membrane, which was probed with specific antibodies. Proteins were visualized using enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, U.K.).

FIG. 2. Expressions of PDGF receptor, signal transduction molecules, GLUTs, and basal activities of these signaling molecules in striated muscles from wild type (WT) and transgenic mice expressing human PDGF receptor (PDGF-R) specifically in skeletal muscles. A: Various muscle homogenates were prepared from soleus muscles, extensor digitorum longus (EDL) muscles, and cardiac muscles (heart), as described under RESEARCH DESIGN AND METHODS. Muscle homogenates (30 μg) were subjected to Western blot analysis with the indicated specific antibodies. The amount of Akt was assessed with anti-Akt antibody, which reacts with three isoforms of Akt (i.e., Akt1, -2, and -3). Anti-β-tubulin blot was also performed as a loading control. B: To investigate the activity of PI 3-kinase in striated muscles at a basal state, immunoprecipitates from muscle homogenates (0.5 mg) were subjected to PI 3-kinase assay, as described under RESEARCH DESIGN AND METHODS. The formation of 3′-phosphorylated phosphoinositides (PI3P) is indicated as the radioactivity in the spots. To investigate the phosphorylation of Akt in striated muscles at a basal state, muscle homogenates (30 μg) were subjected to Western blot analysis with anti–phospho-Akt antibody (Ser473). Each representative experiment and the quantitative analysis (National Institutes of Health Image Program) are shown. C: Quantities of GLUTs in skeletal muscles (gastrocnemius) from wild-type mice and transgenic mice expressing human PDGF receptor specifically in skeletal muscles. Muscle homogenates (30 μg for GLUT4, 40 μg for GLUT1) were subjected to Western blot analysis with anti-GLUT1 or anti-GLUT4 antibody. hetero, heterozygous; homo, homozygous; IB, immunoblot; IP, immunoprecipitate; WT, wild type.
PI 3-kinase assay. Preparation of homogenates from striated muscles was as described above. Equal amounts of protein were incubated with anti-phosphotyrosine antibody (PY99) for 2 h at 4°C, and the immunocomplexes were precipitated with 40 μl of protein A-Sepharose CL-4B and washed twice with each of the following buffers: 1) 20 mmol/l Tris-HCl, pH 8.0, 140 mmol/l NaCl, 1% Nonidet P-40, and 1 mmol/l dithiothreitol; 2) 0.1 mol/l Tris-HCl, pH 7.4, 0.5 mol/l LiCl, and 1 mmol/l dithiothreitol; and 3) 10 mmol/l Tris-HCl, pH 7.4, 100 mmol/l NaCl, and 1 mmol/l dithiothreitol. The immunoprecipitates were subjected to the PI 3-kinase assay in a 50-μl reaction mixture containing 20 mmol/l Tris-HCl, pH 7.4, 50 mmol/l NaCl, 10 mmol/l MgCl₂, 0.5 mmol/l EGTA, 100 μmol/l PI, 100 μmol/l phosphatidyserine, 120 mmol/l adenosine, and 10 μmol/l [γ-32P]ATP (0.1 μCi/μl). After 10 min at 30°C, the reaction was stopped by adding 250 μl of 1 mol/l HCl and 80 μl of chloroform/methanol (1:1, vol/vol). A 30-μl portion of the lower layer was spotted onto a Silica Gel 60 plate (Merck, Darmstadt, Germany) and developed in chloroform/methanol/25% NH₄OH/water (45:35:3:7, vol/vol). The radioactive spot corresponding to phosphoinositide phosphate was visualized autoradiographically.

In vitro incubation system of the skeletal muscle and evaluation of glucose transport. The soleus muscles were isolated from the hindlimbs of mice and then incubated in the indicated solutions according to the in vitro incubation system of the skeletal muscle, as previously described (38), but with some modifications. Briefly, mice were fasted for 16 h before the study and then anesthetized. Excised soleus muscles were incubated in Krebs-Henseleit-HEPES buffer containing 118.5 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 25 mmol/l NaHCO₃, 2.5 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 5 mmol/l L-HEPES, and 0.1% BSA, supplemented with mannitol and glucose or unlabelled 2-deoxyglucose, of which the total concentration was kept at 20 mmol/l to maintain osmolarity. Solutions were continuously exposed to 95% O₂–5% CO₂ to maintain pH 7.4. After pretreatment of the ligand for 30 min, 2-deoxy-D-[1,2-3H]glucose uptakes were then measured by exposing soleus muscles for 15 min to 1.5 μCi of 2-deoxy-D-[1,2-3H]glucose with ligand. Muscles were also exposed to 0.3 μCi of [3H]-mannitol to determine the extracellular volume. After the incubation, muscles were homogenized and the radioactivity of the 2-deoxy-[3H]-glucose uptake was measured, as previously described (38).

RESULTS AND DISCUSSION

Generation and data on transgenic mice specifically expressing PDGF receptor in skeletal muscles. We used a muscle creatine kinase enhancer and promoter of the mouse genome (Fig. 1A) because the mouse has no detectable endogenous PDGF receptor in skeletal muscles (Fig. 2A) (35). We generated five founder transgenic mice expressing human PDGF receptor specifically in skeletal muscles, and two mainly analyzed lines were designated mPDGF·R-TG#2 and mPDGF·R-TG#510. After digestion of the mouse genome by BamHI, 7- and 1-kb fragments of the PDGF receptor transgene in mPDGF·R-TG#2 and mPDGF·R-TG#510 lines were demonstrated by Southern blot analysis using as a probe 3.8-kb human PDGF-β receptor cDNA (Fig. 1A [black section] and B). Analysis of mRNA levels using Northern blots revealed that the PDGF receptor was restrictedly expressed in skeletal muscles (hindlimbs) and heart in proportion to the amount of transgene in mPDGF·R-TG#2 (Fig. 1C). The same results were also obtained with mPDGF·R-TG#510 (data not shown).

As shown in Fig. 2A, Western blot analysis of transgenic mice expressing human PDGF receptor specifically in skeletal muscles with an anti-PDGF receptor antibody demonstrated the presence of the two bands, the upper ones indicating the mature form of human PDGF receptor and the lower ones the precursor, as previously described (40). In contrast, no bands were demonstrated in wild-type mice, which suggested that a detectable endogenous PDGF receptor did not exist in skeletal muscles of these wild-type mice. Exogenously expressed PDGF receptor in skeletal muscles of the transgenic mice was slightly auto-phosphorylated at the basal state in parallel with the amount of PDGF receptor (Fig. 3A). The downstream signaling effectors, i.e., PI 3-kinase and Akt, were also slightly activated in the basal state (Fig. 2B) in proportion to autophosphorylations of PDGF receptor. Akt activity was measured based on the phosphorylation of Ser at 473 of Akt (41). Interestingly, the amount of Akt increased in parallel with the amount of PDGF receptor, especially in extensor digitorum longus muscles (Fig. 2A). On the other hand, there was no detectable difference in expressions of GLUTs, i.e., GLUT4 and GLUT1, among wild-type, mPDGF·R-TG#2 (homozygous), and mPDGF·R-TG#510 (heterozygous) mice as determined by Western blot analysis (Fig. 2C).
FIG. 3. Ligand-stimulated activities of signaling molecules and glucose transport in isolated skeletal muscles from wild-type (WT) mice and transgenic mice expressing human PDGF receptor (PDGF-R) specifically in skeletal muscles. A: Soleus muscles from wild-type mice (15 weeks old), mPDGF-R-TG#2 mice (homozygous; 13 weeks old), and mPDGF-R-TG#510 mice (heterozygous; 19 weeks old) were stimulated by the ligand treatment for 35 min according to the in vitro incubation system of the skeletal muscle, and then they were homogenized with homogenizing buffer. Equal amounts of muscle homogenates were subjected to immunoprecipitation with anti-PDGF receptor antibody and then to Western blot analysis with anti-phosphotyrosine antibody as described under RESEARCH DESIGN AND METHODS. Equal amounts of muscle homogenates were also subjected to Western blot analysis with the indicated specific antibodies. Anti-β-tubulin blot was also performed as a loading control. B: Equal amounts of muscle homogenates were subjected to PI 3-kinase assay, as described under RESEARCH DESIGN AND METHODS, or subjected to Western blot analysis with anti–phospho-Akt antibody (Ser473). Each representative experiment and the quantitative analysis (National Institutes of Health Image Program) are shown. C: Glucose uptake stimulated by the ligand treatment for 45 min in soleus muscles from each genotype mice (9–12 weeks old) was measured, as described under RESEARCH DESIGN AND METHODS. Values represent the means ± SE for 4–11 samples per group. Data were analyzed with one-way ANOVA, and multiple comparison methods of Fisher projected least significant difference were used with a significant level of 0.05, 0.01, or 0.001. *0.05; **0.01; ***0.001. □, basal; ▲, 500 ng/ml PDGF; △, 0.1 mU/ml insulin. hetero, heterozygous; homo, homozygous; IB, immunoblot; Ins., insulin; IP, immunoprecipitate. PI3P, 3'-phosphorylated phosphoinositides.
Ligand-stimulated activities of signaling molecules and glucose transport activity in isolated skeletal muscles. To assess the function of exogenous PDGF receptor in skeletal muscles, soleus muscles were isolated and incubated in solution with or without either PDGF or insulin according to the in vitro incubation system of the skeletal muscle (38). PDGF stimulated autophosphorylations of PDGF receptor in isolated soleus muscles of transgenic mice (Fig. 3A), and as a consequence PI 3-kinase and Akt were dose-dependently activated (Fig. 3B). These signaling molecules, PI 3-kinase and Akt, were also activated by insulin. Next, we examined PDGF-stimulated glucose uptake according to the in vitro incubation system of the skeletal muscle. As shown in Fig. 3C, PDGF treatment for 45 min stimulated glucose uptake intensively in vitro in isolated soleus muscles from mPDGF·R-TG⁵¹⁰ and mPDGF·R-TG⁵₁₀, compared with findings in wild-type mice. On the other hand, insulin-stimulated glucose transport activity did not differ between wild-type mice and transgenic mice expressing human PDGF receptor specifically in skeletal muscles. These findings elucidate that a signaling pathway from the PDGF receptor has the potential to stimulate glucose transport in skeletal muscles of the transgenic mice, and that some of the PDGF signaling molecules are essentially shared with insulin for glucose transport as noted based on experiments with cultured cells (20,21,24–26). In skeletal muscles of mPDGF·R-TG⁵¹⁰ and mPDGF·R-TG⁵₁₀, the basal activities of PI 3-kinase and Akt are slightly higher than those of wild-type mice (Figs. 2B and 3B), but basal glucose uptake in vitro did not differ in these mice (Fig. 3C). PDGF locally secreted from around fibroblasts (42) seems to activate the basal kinase of the PDGF receptor (Figs. 3A and 4A) and basal PI 3-kinase in skeletal muscles (Figs. 3B and 4B). In Figs. 2B and 4B, PI 3-kinase activity was measured in skeletal muscles immediately after dissection, whereas PI-3 kinase activity in Fig. 3B was measured in isolated soleus muscles after incubation for 35 min in the basal buffer, events that may result in attenuated basal PI3K activity in Fig. 3B as compared with that in Fig. 2B and 4B.

Increased PI 3-kinase activity by stimulation of EGF in isolated human skeletal muscles fails to stimulate glucose uptake (43). We and others reported that Akt2 had a pivotal role in glucose transport signaling by insulin (12–14), whereas EGF did not significantly increase Akt2 activity in isolated human skeletal muscles (43). However, we and another group reported that EGF triggers GLUT4 translocation and stimulates glucose uptake in cultured cells (22,23). Therefore, the stimulation of glucose uptake by EGF seems to be controversial. PDGF signaling has the potential to activate Akt2 (32); therefore, the stimulation by PDGF in isolated skeletal muscles from mPDGF·R-TG⁵¹⁰ and mPDGF·R-TG⁵₁₀ might be sufficient for activation of signaling pathways concerning the increase of glucose transport in skeletal muscles. All of these observations on glucose transport by PDGF in isolated skeletal muscles do not contradict the multiple studies in cultured cells (20,21,24–26).

In vivo effects of the intramuscular injection of PDGF in transgenic mice expressing human PDGF receptor specifically in skeletal muscles. To assess the effect of PDGF in transgenic mice in vivo, we examined PDGF receptor autophosphorylation, activations of PI 3-kinase, and Akt after the intramuscular injection of PDGF. PDGF potently stimulated PDGF receptor autophosphorylation, activities of PI 3-kinase, and Akt in skeletal muscles of mPDGF·R-TG⁵¹⁰ (homozygous) and mPDGF·R-TG⁵₁₀ (heterozygous) in vivo (Fig. 4A and B). In contrast, in wild-type mice PI 3-kinase activity and Akt phosphorylation were slightly stimulated, because a very small amount of PDGF receptor of fibroblasts in connective tissue and myoblasts (44) was probably activated. In skeletal muscles from mPDGF·R-TG⁵₁₀ (heterozygous), PDGF pretreatment attenuated the phosphorylation of IRS-1 and Akt and the activity of PI 3-kinase by the stimulation of insulin (T.Y., Y.E., unpublished observations), as previously reported (31). Intravenous injections of various doses of PDGF (0.08–80 μg) instead of intramuscular injection (1 μg) did not give reproducible results for activations of PDGF receptor kinase, PI 3-kinase, and Akt in skeletal muscles of transgenic mice (data not shown). Therefore, PDGF was injected intramuscularly to observe the effects of PDGF in skeletal muscles of transgenic mice.

Next, we examined the insulin- or PDGF-stimulated glucose uptake in skeletal muscles in vivo. After 20 min of intramuscular (bilateral hindlimbs) injection of PDGF or insulin, [³H]glucose was injected into the jugular vein, and glucose uptake into the soleus muscles was measured 20 min later, as described in RESEARCH DESIGN AND METHODS. The basal glucose uptake by soleus muscles was similar among wild-type, mPDGF·R-TG⁵¹⁰ (homozygous), and mPDGF·R-TG⁵₁₀ (heterozygous) mice. PDGF significantly stimulated glucose uptake in soleus muscles of transgenic mice expressing human PDGF receptor specifically in skeletal muscles, and the degree of glucose uptake reached ~60% of that seen with insulin injection (Fig. 4C). We determined whether this metabolic action of PDGF was mediated by IRS-1. In skeletal muscles from both wild-type mice and transgenic mice expressing human PDGF receptor specifically in skeletal muscles, only insulin injection elicited tyrosine phosphorylation of IRS-1 (Fig. 4D), which was identical with the results in 3T3-L1 adipocytes (26). We suggest that PDGF injection in vivo might decrease...
blood glucose levels because muscle glucose uptake can account for >80% of the total amount of blood glucose metabolized after insulin injection (4,45,46). As shown in Fig. 2C, both GLUT1 and -4 are expressed in skeletal muscles. We reported that PDGF stimulates GLUT1 gene expression and consequently increases glucose uptake (21,47), but we found no difference after PDGF injection in the expression of GLUTs during the experiments (Fig. 4E).

PDGF-stimulated glucose transport was reported to occur by the translocation of GLUT1 instead of GLUT4 (27,29).
FIG. 4. Continued

C

\[2\text{HJDG Uptake (min}^{-1}\text{)}\]

WT     mPDGF-R-TG\#2 (homo)    mPDGF-R-TG\#510 (hetero)

**  

**  

**  

Control

1 \mu g PDGF

0.5 U/kg Insulin

D

IP : anti-IRS1

IB : PY99

IP : anti-IRS1

IB : anti-IRS1

Phospho-IRS-1

IRS1

E

WT     mPDGF-R-TG\#2 (homo)    mPDGF-R-TG\#510 (hetero)

PDGF

+   -   +   -   +   -

IB: anti-GLUT4

IB: anti-GLUT1

GLUT4

GLUT1

F

WT

Blood Glucose (mg/dl)

Minutes after Injection

G

mPDGF-R-TG\#2 (homo)

Blood Glucose (mg/dl)

Minutes after Injection

H

mPDGF-R-TG\#510 (hetero)

Blood Glucose (mg/dl)

Minutes after Injection

FIG. 4. Continued
However, we and others reported that PDGF triggers GLUT4 translocation and stimulates glucose transport in cultured cells (20,21,24–26). We previously constructed 3T3-L1 adipocytes, which stably express myc epitope–tagged GLUT4 and -1, to estimate precisely translocations of these GLUTs on the cell surface by PDGF (21). We found that PDGF induces a fivefold increase of GLUT4 translocation but a twofold increase of GLUT1 translocation in these 3T3-L1 adipocytes (21). Therefore, a 4.5-fold increase in glucose transport by PDGF in skeletal muscles of transgenic mice expressing human PDGF receptor specifically in skeletal muscles (Fig. 4C) seems to be caused not only by GLUT1 translocation but also by GLUT4 translocation. Because, in extensor digitorum longus muscles, the stimulation of glucose uptake by insulin in vivo was much lower (less than twofold) than in soleus muscles (more than severalfold) (T.Y., Y.E., unpublished observations; and 48), PDGF did not show a significant effect on glucose uptake in extensor digitorum longus muscles (data not shown).

Finally, we assessed the blood glucose–lowering effect of exogenously injected PDGF in these transgenic mice expressing human PDGF receptor specifically in skeletal muscles. Intramuscular injection of insulin leads to a rapid decrease in blood glucose levels in wild-type mice (Fig. 4F) and in both mPGF·R-TG82 and 510 (Fig. 4G and H). However, the intramuscular injection of PDGF in these mice does not affect the blood glucose level (Fig. 4F, G, and H), even though PDGF stimulates glucose uptake in soleus muscles in these transgenic mice expressing human PDGF receptor specifically in skeletal muscles (Fig. 4C). The intramuscular injection of PDGF in the whole body (bilateral hind and forelimbs) did not affect the blood glucose level (data not shown). Although skeletal muscles are the major tissues responsible for insulin-stimulated blood glucose disposal (>80% of blood glucose) (4,45,46), insulin regulates glucose metabolism not only in skeletal muscles but also in liver and in adipose tissues in order to accurately maintain glucose homeostasis. In fact, mice specifically lacking GLUT4 in adipocytes had an impaired glucose-lowering effect by insulin, even though basal and insulin-stimulated glucose uptake in isolated skeletal muscles are comparable to findings in control mice (49). On the other hand, insulin injection decreased blood glucose levels in muscle-specific insulin receptor knockout mice (50), but not in liver-specific insulin receptor knockout mice (51). These studies on knockout mice suggested that a considerable portion of the decline in blood glucose levels after administration of insulin is caused by suppression of hepatic glucose output, and it is partly caused by glucose uptake in adipose tissues, rather than an increase in muscle glucose disposal. In conscious dogs, insulin rapidly suppresses hepatic glucose output and augments net hepatic glucose uptake via both direct and indirect effects (52,53).

Based on these studies, we propose the hypothesis that augmentation in muscle glucose disposal alone might not fully elicit a rapid decline in blood glucose levels. We found the tyrosine phosphorylation of the insulin receptor β-subunits was inhibited in skeletal muscles from intramuscularly insulin-injected mPGF·R-TG8510, using a Western blot analysis after immunoprecipitation by anti-phosphotyrosine antibody (T.Y., Y.E., unpublished observations), which was comparable with the results seen in Fig. 4A. This inhibition of insulin signaling seems to account for the impaired glucose-lowering effect during the time just after insulin injection (from 5 to 15 min) in transgenic mice expressing human PDGF receptor specifically in skeletal muscles (Fig. 4G and H).

The degree of glucose uptake in vivo reached ~60% of that by insulin injection in skeletal muscles (Fig. 4C). This may be caused by an inappropriate cellular compartment of signals or an insufficient amount of PI-3,4,5-trisphosphate by PDGF stimulation (54), or the PI 3-kinase activation pathway alone may not be sufficient to mediate the full effect on glucose transport. It has been reported that an alternative, parallel pathway is implicated in insulin-stimulated glucose transport (19). The major controversy concerning the signaling is whether compartmentalization of the signal or the amount of PI-3,4,5-trisphosphate that is generated is the cause of the specificity of insulin action on glucose transport. It is possible that the activity of PDGF receptor is regulated by its targeting to lipid raft domains in the plasma membrane, as in the case of the activity of the EGF receptor (55), and that some of the overexpressed PDGF receptors are localized incorrectly and activated in the basal state (Figs. 3A and 4A). We reported that the effects of PDGF on the activation of PI 3-kinase and the stimulation of GLUT4 translocation and glucose uptake are transient in 3T3-L1 adipocytes, which have relative low levels of endogenous PDGF receptor (21). Tengholm and Meyer (54) also reported that the transient production of PI-3,4,5-trisphosphate by PDGF stimulation was not sufficient to retain GLUT4 in the plasma membrane. These observations can explain the difference in glucose uptake in vivo stimulated by insulin and by PDGF. PDGF does not seem to efficiently activate all of the essential signaling molecules required for glucose transport by insulin.

ACKNOWLEDGMENTS

This work was supported by research grants from the Ministry of Education, Science, Technology, Sports, and Culture of Japan (to K.K., T.O., and Y.E.); the Kowa Life Science Foundation; the Mitsui Life Social Welfare Foundation; and the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to K.K.).

We thank Dr. J. Chamberlain and Dr. S. Hauschka (University of Washington, Seattle, WA) for kindly providing the muscle creatine kinase–6.5 kb enhancer/promoter/first intron segment, Dr I. Nishino (National Institute of Neuroscience National Center of Neurology and Psychiatry, Tokyo) for histological analysis of skeletal muscles, Dr. Y. Kawano (Ohtsuka Pharmaceutical, Tokyo) for technical advice on glucose uptake into isolated skeletal muscles, and I. Miyata for technical assistance.

REFERENCES


